

TAXONOMY OF *JUNIPERUS COMMUNIS* IN NORTH AMERICA: INSIGHT FROM VARIATION IN nrDNA SNPs

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ABSTRACT

Plants of *Juniperus communis* L. var. *communis*, *J. c.* var. *depressa* Pursh, *J. c.* var. *jackii* Rehr, *J. c.* var. *saxatilis* Pall. were sampled and SNPs from nrDNA were examined. Based on these data and previous data, a new variety of *J. communis* is recognized: *Juniperus communis* var. *charlottensis* R. P. Adams, var. nov. It occurs in muskeg bogs on Queen Charlotte Island, British Columbia and in the Ketchikan, Alaska area. *Juniperus communis*, in North America, is treated as five varieties: vars. *charlottensis*, *depressa*, *jackii*, *megistocarpa*, and *saxatilis*. Keys and distribution maps are presented.

KEY WORDS: *Juniperus communis*, *J. communis* var. *charlottensis*, var. *depressa*, var. *jackii*, var. *megistocarpa* and var. *saxatilis*, Cupressaceae, geographic variation, nrDNA, SNPs.

Juniperus communis is the only *Juniperus* that occurs in both the eastern and western hemispheres. In North America, *J. communis* has been treated (Adams, 2004) as composed of as many as four varieties. More recently, Adams and Nguyen (2007) treated the north American *J. communis* as three varieties: *J. c.* var. *depressa*, *J. c.* var. *jackii*, and *J. c.* var. *megistocarpa* (Fig. 1). Their classification was based on RAPDs data and leaf morphology.

Analysis of Arctic populations of *J. communis* (Adams et al., 2003) revealed that these populations clustered by continent, with the populations in Greenland and Iceland showing the highest affinities to populations from Europe and not to those from North America (Fig. 1).

Adams et al. (2003) concluded that the post-Pleistocene populations on Greenland and Iceland came from Europe and not North America. Unfortunately, none of the Pacific northwestern putative *J. c.* var. *saxatilis* was included in the study.



Figure 1. Minimum spanning network showing that the North American *J. communis* var. *depressa* and var. *megistocarpa* populations link together and all the *J. communis* populations from the e. hemisphere link together (Adams et al., 2003). The dashed line is the minimum link between eastern and western hemisphere populations.

Analysis of the currently named *J. communis* varieties (Adams and Pandey, 2003), resolved these taxa into six major groups: *J. c.* var. *communis* and var. *saxatilis* Pall. from Europe; *J. c.* var. *depressa*, N.

America; *J. c.* var. *megistocarpa*, Quebec; *J. c.* var. *nipponica* (Maxim.) E. H. Wilson, Japan; and putative *J. c.* var. *saxatilis*, Kamchatka, Russia. However, Adams and Pandey (2003) did not include *J. c.* var. *jackii*, or putative *J. c.* var. *saxatilis* from the Pacific northwest, US/Canada in their analysis.

Ashworth, et al. (1999, 2001) used DNA fingerprinting to examine *J. communis* plants identified as *J. c.* var. *depressa*, *J. c.* var. *jackii*, *J. c.* var. *montana* Aiton (= *J. c.* var. *saxatilis*, see Adams, 2004) collected from California, Oregon, Nevada and Utah in the southwest and west coast of the United States. They did not get a clear pattern separating these taxa, and concluded that their samples represented a single varietal taxon. However, it not clear if they utilized replicated samples to remove spurious variation in RAPD bands.

Adams and Nguyen (2007) collected additional samples of putative *J. c.* var. *saxatilis* from the Pacific northwest, *J. c.* var. *jackii* from NW California and *J. c.* var. *depressa* from the southernmost locations in North America (Mt. Charleston, Nevada and Mt. Satula, North Carolina).

The major trend (figure 2) among the taxa was the separation of the eastern hemisphere plants (*J. communis* var. *communis*, *J. c.* var. *saxatilis*, and putative *J. c.* var. *saxatilis*, Kamchatka) from the western hemisphere plants (*J. c.* var. *depressa*, *J. c.* var. *jackii*, *J. c.* var. *megistocarpa*, and putative var. *saxatilis*). The resolution (figure 2) of *J. c.* var. *jackii* (and plants from Mt. Hood) was in contrast to the report by Ashworth, et al. (1999, 2001). The Banff, Alberta individuals (putative hybrids) were intermediate between the coastal, short, curved-leaved plants (putative var. *saxatilis*) and *J. c.* var. *depressa* (figure 2). *Juniperus c.* var. *megistocarpa* was distinct from *J. c.* var. *depressa*.

However, examination of additional *J. communis* specimens from California, Oregon, Washington, British Columbia and Alaska revealed that plants with short, curved leaves and a stomatal band 1.5 to 2 times as wide as the green side bands are common in the Pacific Northwest to Alaska and even eastward into Idaho at Redfish Lake and Banff, Alberta. To further elucidate the patterns of variation in *J. communis*, an analysis involving sequencing the ITS of nrDNA was conducted. The purpose of the present study is to present new data and

combine this information with previous results to help resolve the very complex patterns of variation in *J. communis* from North America.

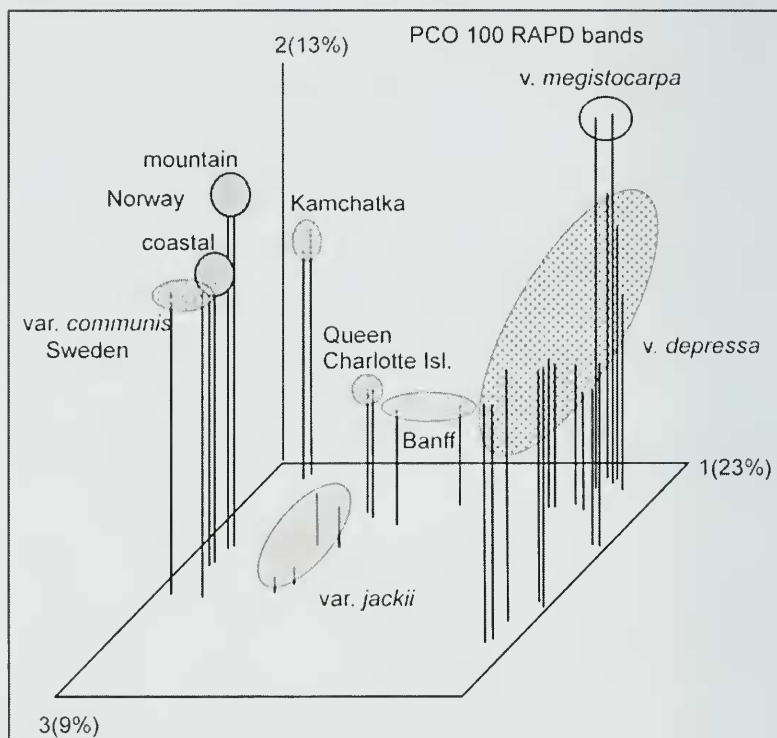


Figure 2. PCO based on 100 RAPD bands (Adams and Nguyen, 2007).

MATERIALS AND METHODS

Specimens used in the present study: *J. communis* var. *communis*: Adams 11173-11175, Norway; *J. c.* var. *depressa*: Adams 7582-7584, Denali National Park, AK, USA; Adams 9394-9395, Hudson Bay, Quebec, Canada (ex N. Dignard); Adams 10225-10229, on granite, Mt. Satula, NC, USA; Adams 7802-7804, Victor, CO, USA; Adams 11230-11232, Vancouver Island, BC, Canada (ex. A. Ceska); Adams 11128-11132, Ellery Lake Dam, CA, USA; Adams 11233-

11235, 11237-11238, Coastal Range, n BC, Canada (ex Ken Marr); *Adams* 11236, Goat Lake, Olympic National Park, WA, USA (ex Ken Marr); *Adams* 11074, sand dunes, Whidbey Island, WA, USA; *Adams* 8572-8574, MA, USA; *Adams* 10225-10229, on Granite, Mt. Satula, NC, USA, *J. c.* var. *jackii*: *Adams* 10287-10291, serpentine, Del Norte Co., CA; USA, *Adams* 10300-10303, on volcanic rock, Mt. Hood, Wasco Co., OR, USA; *J. c.* var. *saxatilis*: *Adams* 11206, 11207 Norway; *Adams* 8685 - 8787, Hokkaido, Japan, *Adams* 10890-10893, Redfish Lake, Idaho; *Adams* 10304-10308, Queen Charlotte Island, BC, Canada; *Adams* 9181-9183 Eso, Kamchatka, Peninsula, Russia (ex. J. W. Leverenz). Voucher specimens are deposited at the Baylor University herbarium (BAYLU).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia CA).

SNPs obtained from DNA sequencing

ITS (nrDNA) amplifications were performed in 50 µl reactions using 10 ng of genomic DNA, 3 units Qiagen Taq polymerase, 5 µl 10x buffer [final concentration: 50 mM KCl, 10 mM Tris-HCl (pH 9), 0.01% gelatin and 0.1% Triton X-100], 1.75 mM MgCl₂, 20 µl Q solution (2X final), 400 µM each dNTP, 1.8 µM each primer and 4% (by vol.) DMSO].

Primers (5'-3'):

ITS: ITSA = GGA AGG AGA AGT CGT AAC AAG G;

ITSB = CTT TTC CTC CGC TTA TTG ATA TG.

ITSA and ITSB primers from Blattner (1999).

The following PCR conditions were used: MJ Research Programmable Thermal Cycler, 30 cycles, 94°C (1 min.), 50°C (2 min.), 72°C (2 min.), with a final step of 72°C (5 min.). The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). The nrDNA primers (ITSA, ITSB) produced a band of approx. 1120 bp. In each case the band was excised and purified using a Qiagen QIAquick gel extraction kit.

The gel purified DNA band with the appropriate primer was sent to McLab Inc. for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments were made using Clustal W and then manually corrected. Indels were coded with a "-" for the first nucleotide and "I" for succeeding nucleotides such that an indel was treated as a single mutation event. Overall sequences have been deposited in GenBank (Schwarzbach et al., in prep.).

SNPs analyses

The aligned data sets were analyzed by CLEANDNA (Fortran, R. P. Adams) to remove invariant data. Mutational differences were computed by comparing all SNPs, divided by the number of comparisons over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). A minimum spanning network was constructed by selecting the nearest neighbor for each taxon from the pair-wise similarity matrix, then connecting those nearest neighbors as nodes in the network (Adams, 2004).

RESULTS AND DISCUSSION

Analyses of 1119 bp of nrDNA (ITS) sequences revealed 23 SNPs among the taxa including 2 and 4 bp deletions in *J. c. var. communis* and *var. saxatilis* from Norway as well as *J. c. var. jackii* (NW CA and Mt. Hood, OR). The 2 bp and 4 bp deletions were each coded as single deletion events in making comparisons.

Factoring the association matrix yielded five eigenroots before they began to asymptote, implying that six groups were present. These eigenroots accounted for 48.9, 15.8, 12.0, 6.9, and 5.7% of the variation among the 58 individuals analyzed. PCO of the eigenvectors (Fig. 3) clearly shows that the differentiation of *J. c. var. jackii* from NW CA and Mt. Hood, OR accounts for 49% of the variance among the 58 individuals. *Juniperus c. var. jackii* had 5 bp differences, plus a 4 bp deletion and a 2 bp deletion. Interestingly, the 4 bp and 2 bp deletions were shared with *J. c. vars. communis* and *saxatilis* from Norway. The Queen Charlotte Island junipers are separated by 2 bp (Fig. 3). These

junipers grow in muskeg bogs that are very atypical of *J. communis*. An individual plant of *J. communis* found growing on a sand dune on Whidbey island has 3 mutations that separate it from all other individuals (Fig. 3). The leaves and habit of the plant are similar to *J. c. var. depressa*. All of the other samples (27 individuals) of *J. c. var. depressa* (N. A.) and *v. saxatilis* (Asia) form a fifth group.

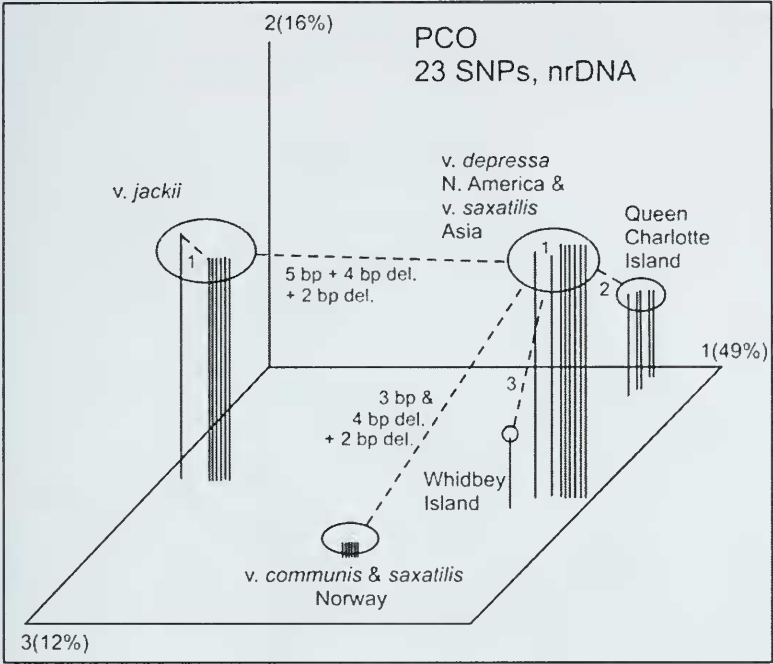


Figure 3. PCO of 58 individuals based on 23 SNPs. Identical bars, closely spaced indicate no variation among these individuals. The bars in the largest group are symbolic, as that group contains 27 individuals!

Because six groups were indicated by the presence of five significant eigenroots, additional variation is hidden in the PCO in figure 3. Therefore, the *J. c. var. jackii* individuals (NW CA and Mt. Hood, OR) were removed and the PCO re-run. This resulted in four eigenroots accounting for 35.5, 23.7, 13.4 and 11.0% of the variance

before asymptoting. This implies the presence of five groups as seen in figure 4. Each of the groups is separated by 2 - 3 bp (plus 4 bp and 2 bp deletion in the Norway group). The large group (27 individuals) of *J. c. var. depressa* and *J. c. var. saxatilis* is relative uniform, with only 1 bp difference between members of that group. All of the individuals that morphologically appear to be *J. c. var. saxatilis* (short, curved leaves, with a stomatal band that is twice as wide the green leaf margins) are within the large group. Therefore, the addition of 'saxatilis' plants from NW North America to this analysis did not change the PCO in comparison with the RAPDs data (Fig. 2).

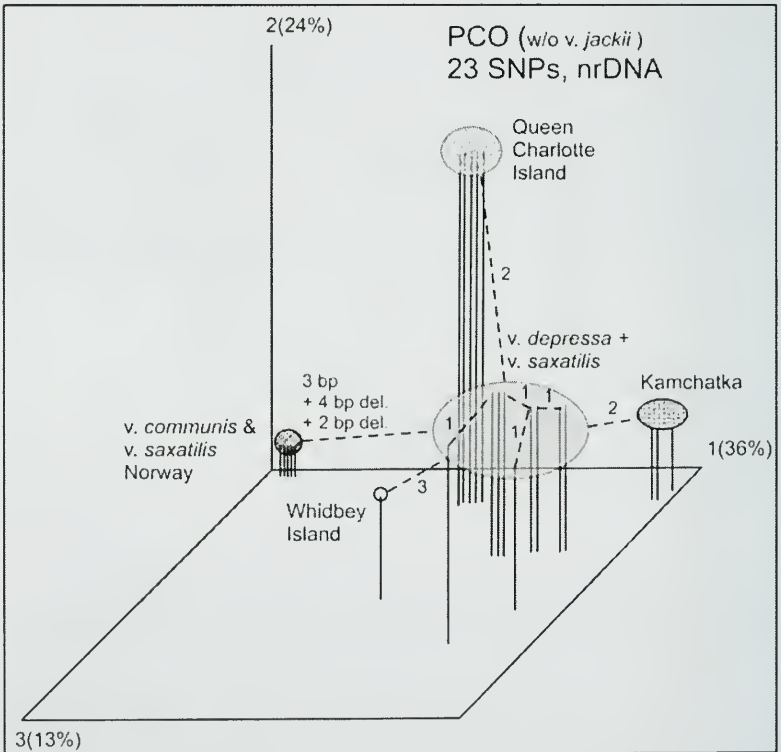


Figure 4. PCO of 23 SNPs of nrDNA without *J. c. var. jackii* individuals.

However, it is interesting that *J. c.* var. *saxatilis* from Japan is within this group, in contrast to a previous RAPDs study (Adams and Nguyen, 2007). Differentiation in the nrDNA sequence was not as great as found in RAPDs. However, the pattern of considerable differentiation in the RAPDs of the *J. c.* var. *jackii* and Queen Charlotte Island plants (Fig. 2) is clearly concurrent with the nrDNA (Figs. 3, 4).

An unusual aspect of this study of nrDNA was the association of mutations across widely spaced distances. Figure 5 shows the first group with six widely distributed individuals with identical nrDNA.

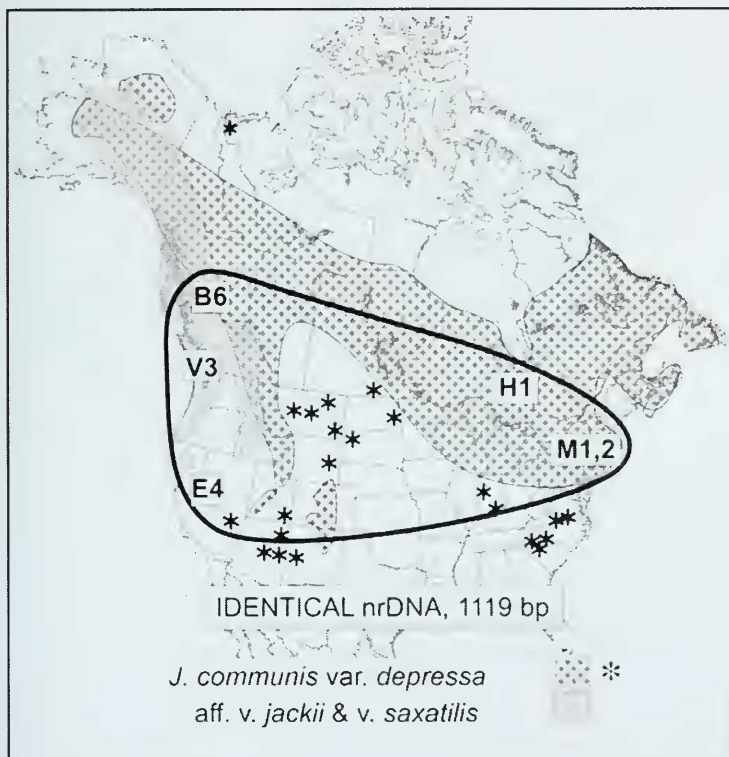


Figure 5. Six individuals, widely spaced, with identical nrDNA.

Each of the six plants shows some morphological affinity to var. *depressa*, but has one or two nucleotide differences that separate them from var. *depressa*.

Figure 6 depicts the largest group of individuals (20 samples) with identical nrDNAs. The southernmost populations were generally very uniform. This nrDNA pattern is that characteristic of *J. c. v. depressa*. But the California population at Ellery Dam (E1, 2, 3), had a 4th plant that grouped with more northerly plants

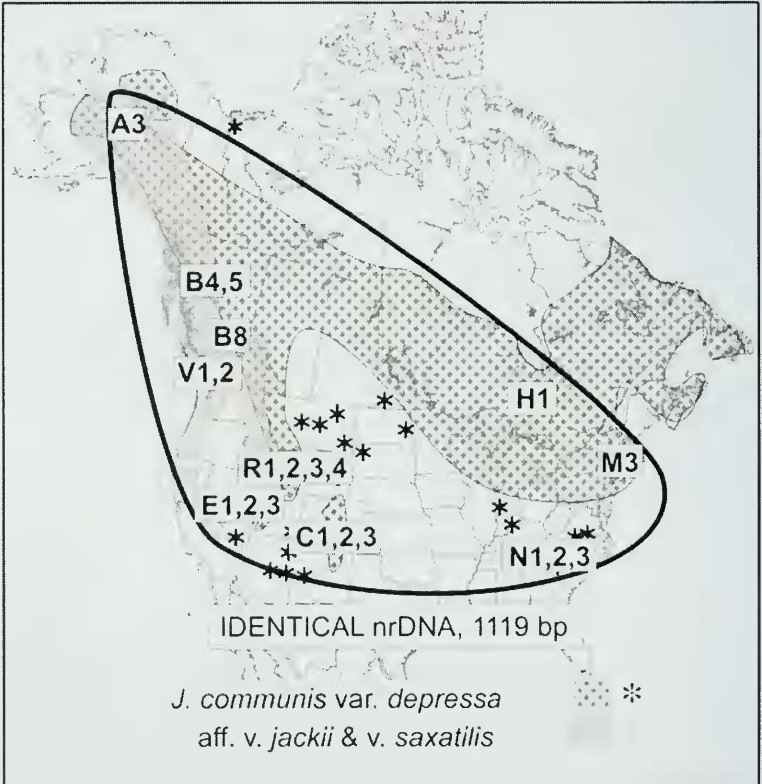


Figure 6. A group of 20 individuals, widely distributed, yet with identical nrDNA sequences.

(Fig. 5). The North Carolina collections (Mt. Satula, N1,2,3) had another individual (N4) that proved identical to *J. c.* var. *saxatilis* from Japan (Fig. 7).

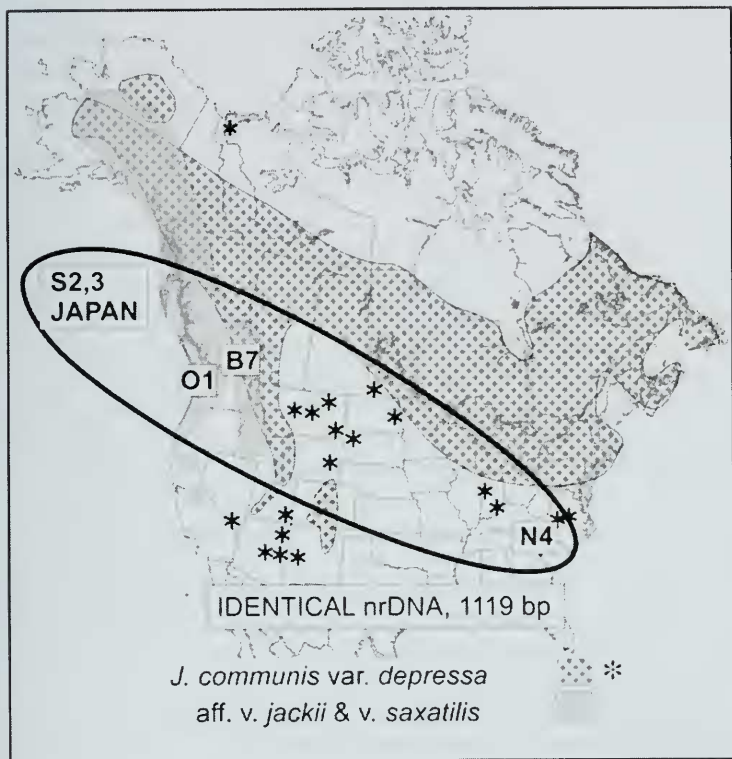


Figure 7. Five individuals with identical nrDNA sequences. Notice that one of the plants from North Carolina (N4) is identical to *J. c.* var. *saxatilis* plants from Japan.

These widely spaced nrDNA identities may reflect a mixing of genotypes after the Pleistocene glaciations. However, it is surprising that such genotypes have persisted in such widely disjunct populations.

TAXONOMIC TREATMENT

The recognition of infraspecific taxa of *J. communis* in North America is a difficult problem. Adams and Nguyen (2007) recognized *J. c. var. depressa*, *J. c. var. jackii* and *J. c. var. megistocarpa* based on leaves, female cones, and RAPDs data. Although the Queen Charlotte Island plants were quite distinct in their RAPDs and their habitat, it was felt that an analysis of plants from the British Columbia mainland were needed before a decision regarding their taxonomic status could be made.

The present study has shown that the Queen Charlotte Island junipers are distinct in their nrDNA from the junipers on the mainland of British Columbia. These junipers grow in a muskeg bog that is atypical of *J. communis*. Undoubtedly, this isolated population has evolved some physiological genes that enable it to cope with this environment. Thus, in addition to divergence in RAPDs and nrDNA, there is also divergence in its physiology. It seems, therefore, appropriate to recognize the plants growing on muskegs on Queen Charlotte Island (and elsewhere) as a new variety:

Juniperus communis var. *charlottensis* R. P. Adams, **var. nov.** TYPE: Canada, Queen Charlotte Island, 9 km s of Masset, along hwy 16, in muskeg bog, 53° 55.511'N, 132° 06.471'W, 61m, 8-July-2007, R. P. Adams 10306 (HOLOTYPE: BAYLU, PARATYPES: R. P. ADAMS 10304, 10305, 10307, 10308 (BAYLU).

Junipero communi var. *jackii* similis sed differt strobilis majoribus globosisque (vs. elongati-subglobosis in var. *jackii*).

This new variety is similar to *J. communis* var. *jackii* but differs in having seed cones that are larger and globose (vs. elongated-subglobose). It was first discovered on Queen Charlotte Island, but examination of specimens from the Ketchikan, Alaska area and islands adjacent to Queen Charlotte Island (see below) revealed that it grows on muskegs in several coastal areas. At present, the habitat seems conserved, so it does not appear to be threatened nor endangered.

ADDITIONAL SPECIMENS EXAMINED: **UNITED STATES, ALASKA, Chichagof Island**, about 0.5 mile NW of Whitestone Harbor, on muskeg, 9 June 1981, *Mary Clay Muller 4257* (ALA), **Mitkof Island, Petersburg**, between downtown and the airport, on muskeg, 12 Feb. 1981, *Mary Clay Muller 4199* (ALA), **Ketchikan area**, near Ward Lake, on muskeg, 15 June 1963, *B. J. Neiland 749* (ALA), **Misty Fjords National Monument, Smeaton Bay area**, Bakewell Trail, on muskeg, 17 July 1980, *D. E. Bramlet B-131* (ALA), **Cleveland Peninsula, Frosty Bay**, on muskeg with sphagnum and sedges, 21 July 1982, *J. Ver Hoef 642* (ALA), **Nakat Inlet**, 2 miles s of head inlet, in Nootka cedar/shore pine on muskeg, 8 July 1993, *J. DeLapp & M. Duffy 93-362* (ALA), **Misty Fjords National Monument, Princess Bay**, e side, in muskeg on exposed rock, 17 July 1993, *J. DeLapp & M. Duffy 93-548* (ALA). **CANADA, BRITISH COLUMBIA, Khtada swamp**, 60 miles sw of Terrace, 18 June 1966, *G. Mendel 196* (V), **Calvert Island, Kwakshua**, on muskeg, 14 July 1939, *I. McT. Cowan ns* (V), **Queen Charlotte Island, Langara Island**, sphagnum bog, 21 May 1952, *F. L. Beebe ns* (V), **Dewdney Island**, w of Pemberton Bay, on rock outcrop in bog, 10 July 1984, *R. T. Ogilvie & Hans Roemer 8471067* (V), **Vancouver Island, Green Mountain**, 35 degree slope, rocky, 1431 m, 16 July 2002, *R. Hebda, N. Hebda, L. Kennedy 02-57* (V).

The distribution of var. *charlottensis*, as presently known, is shown in figure 8. It is interesting that this variety seems to be confined to muskeg bogs that are low-lying, near the ocean. All of the specimens examined grew on muskeg or sphagnum bogs, except the unusual specimen from Green Mountain, Vancouver Island that grew on rocks at 1431 m; whether this specimen is truly var. *charlottensis* is questionable.

The area of distribution of var. *charlottensis* was glaciated during the Wisconsin (Flint, 1971). Because the variety seems restricted to muskeg bogs, it is difficult to determine a refugium for this taxon during the late Pleistocene Wisconsin glacial maximum.

In contrast to our previous study (Adams and Nguyen, 2007, Fig. 2, above), the present study included putative 'var. *saxatilis*' plants from NW US and W Canada. The nrDNA SNPs analyses indicate

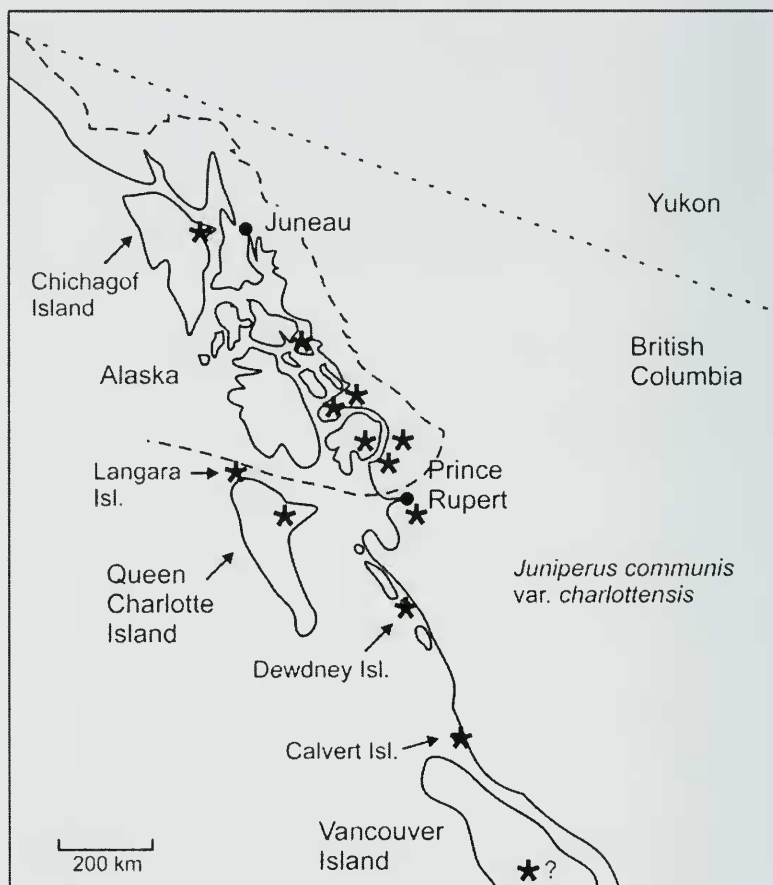


Figure 8. Distribution of *Juniperus communis* var. *charlottensis*. The star with a ? on Vancouver Island is the only location that putative var. *charlottensis* does not grow in a muskeg bog, but in a rocky area.

(Figs. 3, 4) that the putative 'var. *saxatilis*' plants from North America and var. *saxatilis* from the eastern hemisphere are very similar.

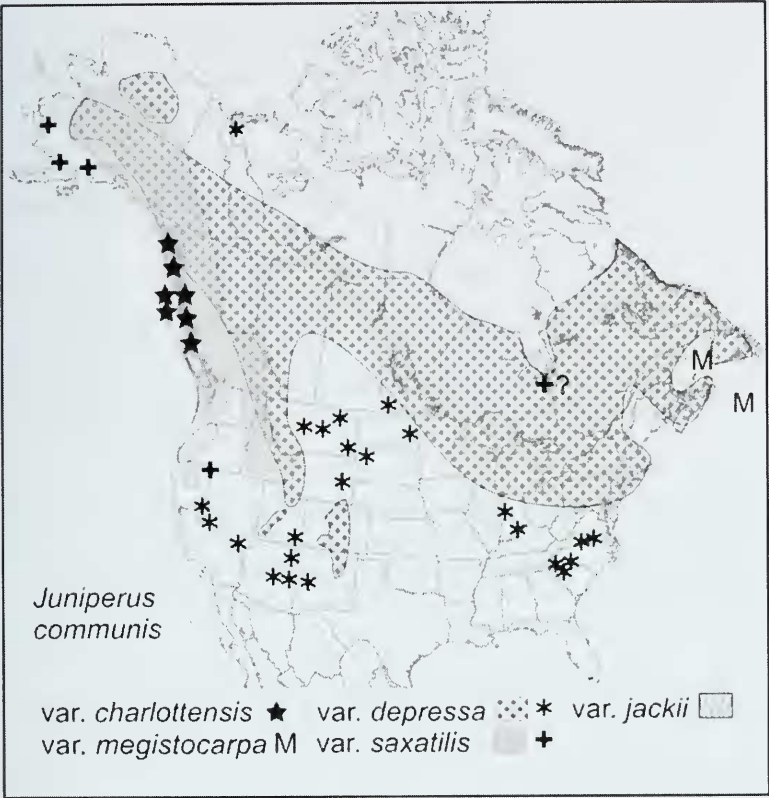


Figure 9. Distribution of *Juniperus communis* varieties in North America. The asterisk and plus symbols represent outlying individuals.

At present, it appears that five (5) varieties of *J. communis* merit recognition in North America: var. *charlottensis*, var. *depressa*, var. *jackii*, var. *megistocarpa*, and var. *saxatilis*. The distributions of the varieties, as presently understood, are shown in figure 9.

Clearly, infraspecific variation in *J. communis* is very complex. The present study has not completely resolved the complex variation. Additional, more detailed populational analysis is being conducted to more fully understand the patterns of variation.

Key to *J. communis* varieties in North America:

1. Glauous stomatal band twice (or more) as wide as each green-leaf margin; spreading, mat-like shrub (or occasionally upright); leaves upright, sometimes almost imbricate, closely set, curved, 5 - 10 (12) mm long.....3.
1. Glauous stomatal band about as wide to 1.5 x as wide as each green-leaf margin; prostrate or low shrub with ascending branchlet tips (or occasionally a spreading shrub), leaves upturned, rarely spreading, linear to curved, 10-20+ mm long.....2.
2. Seed cones 6 – 9 mm diam., smaller than leaf-length; widespread in North America.....var. **depressa**
2. Seed cones 10 – 13 mm diam., larger than leaf length, known only from southeastern Canada.....var. **megistocarpa**
3. Mature seed cones length greater than leaf-length; on muskeg bogs, Calvert Island to Queen Charlotte Island, and north to Chichagof Island, Alaska.....var. **charlottensis**
3. Mature seed cones length less than or equal to leaf-length, Coastal range of w. Canada and U. S., on serpentine, lava and other rock substrates.....4.
4. Mature seed cones elongated-subglobose, stomatal band 3 to 4 times as wide as each green-leaf margin.....var. **jackii**
4. Mature seed cones globose, stomatal band 2 times as wide as each green-leaf margin.....var. **saxatilis**

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